Project No. _____
Book No. 0191

ITLE Exhibit A

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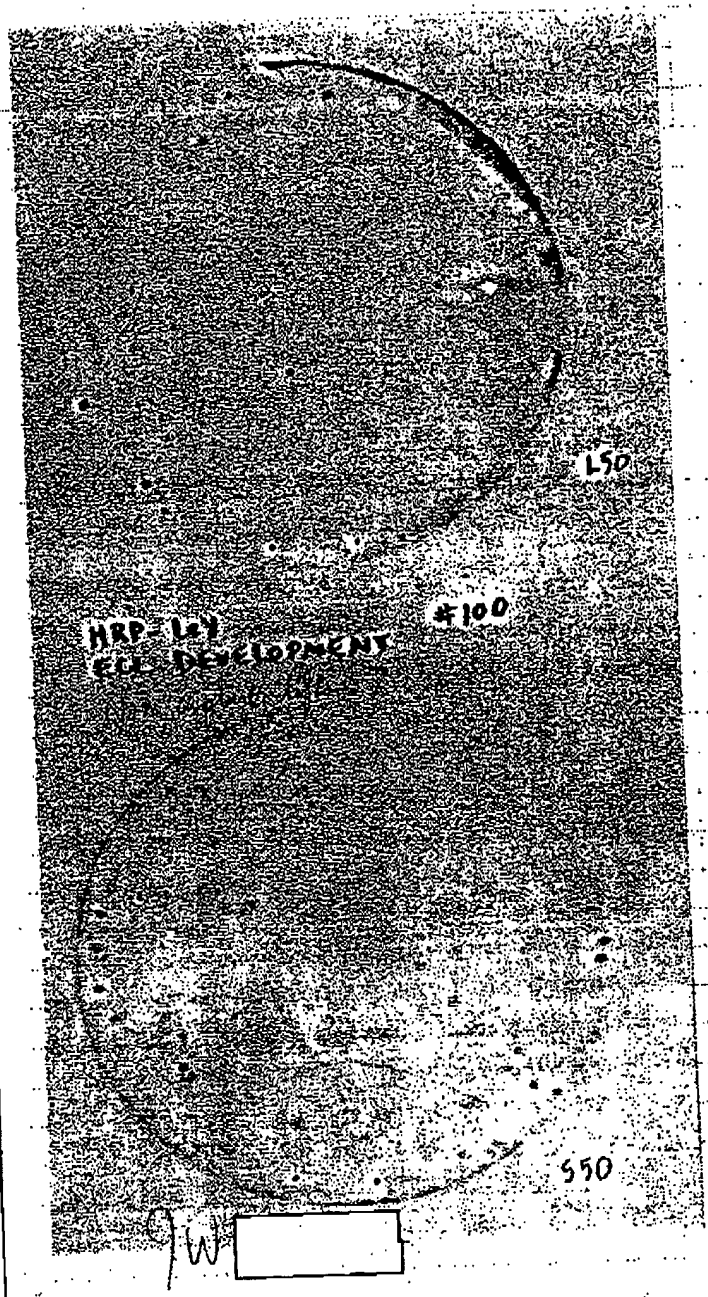
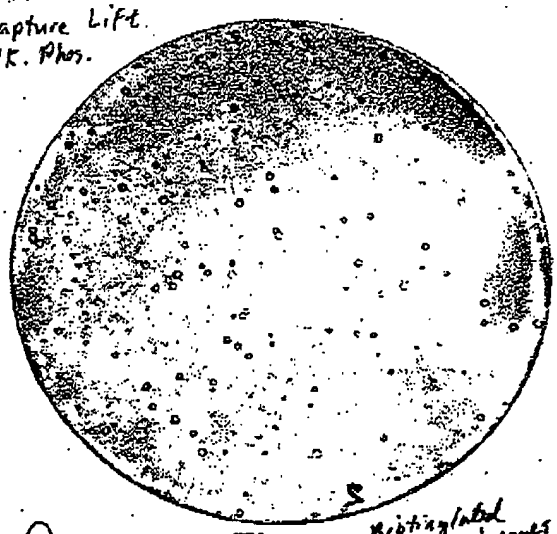
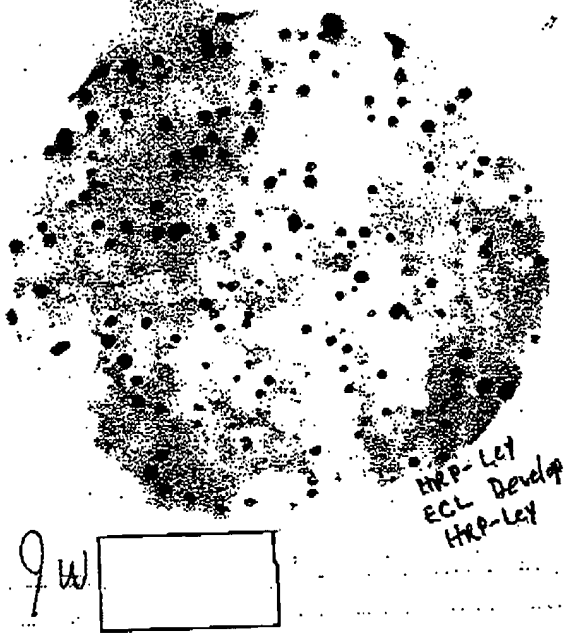


Capture Lift
Alk. Phos.





To Page No.

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Date

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DATE:

TITLE: Filter Lift of Lymph Node Library "Spiked" with 240H2

EXPERIMENT #: 100

BACKGROUND/PURPOSE: In experiment #094 I was able to demonstrate specific binding of my membrane vesicles to 240H2 while having negligible binding to IX64. Today I want to examine the extent of background binding to phage expressing FAb. To do this, I will probe a library expressing human IgG1/k antibodies which has been spiked with 240H2.

DESIGN:

1. Capture lifts were prepared as described previously (#092).

2. The estimated titer of the node library was: 2.3×10^{12} phage/ml.
The estimated titer of the 240H2 stock is: 5.1×10^{11} phage/ml. Essentially the stocks are of the same titer. Therefore, both stocks will be diluted 10^8 (add 90 µl of library to 10 µl of 240H2 into 10 ml of PBS; dilute this stock 1000X twice for the final dilution of 10^8-fold). Plate 2, 10, and 50 µl of this dilution.

3. Based on plaque counts (L2=42; S2=31) the estimated starting titer was between 1.6 and 2.1 $\times 10^{12}$ phage/ml, which is in agreement with the expected values. L=library; S=spiked library.

4. Nitrocellulose filters were placed on L50 and S50 by the normal protocol. These filters were removed 8 hrs later and developed with HRP-LeY by the ECL method.

5. A second filter (capture filter) was placed on the S50 plate and left on ON/RT.

6. Add the following components together:

0.4 ml	biotinylated membranes
0.284 ml	10% TX-100
3.29 ml	1% BSA/PBS/azide
0.029 ml	20% SDS

7. The filter was removed from the plate, rinsed, and placed in the membrane solution described above. The filter was incubated with the membranes 5.5 hrs/4°C. The filter was rinsed briefly with PBS/0.1% Tween 20 under vacuum, and transferred to 10 ml of a 1:1000 dilution of streptavidin alkaline phosphatase in 1% BSA/PBS/azide. The filter was incubated in this solution for 45 min/RT, was washed, and developed 15 min.

8. The first set of lifts, removed for purposes of analyzing HRP-LeY reactivity, did not work very well. Therefore, a second lift was placed on the S50 plate. This filter was analyzed for HRP-LeY reactivity by the ECL method.

RESULTS:

1. A portion of the plaques were reactive with the biotinylated membranes. These same plaques were also reactive with the HRP-LeY reagent. Therefore, the current conditions appear to display specific binding...

2. It also appears as though there are a significant number of HRP-LeY reactive plaques in the L50 sample. It remains to be demonstrated that these reactive plaques are NOT contaminants of BR96 clones from the lab. Along these lines, Paul previously demonstrated that there was 1 contaminant in the library. This was accomplished by screening less than 100 random picks from the library with the peri-prep device. At that time we did not know the source of the contamination (library vs picking stage).

3. Clearly, the capture lift format offers greater sensitivity than previously obtained...

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